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ProductInformation

Thioredoxin Reductase
from rat liver

Product Number **T 9698**
Storage Temperature –20 °C

E.C. 1.8.1.9 (formerly 1.6.4.5)
Synonyms: TrxR; NADPH:oxidized thioredoxin oxidoreductase; thioredoxin: $NADP^+$ oxidoreductase

Product Description
Thioredoxin reductase (TrxR) is an NADPH-dependent oxidoreductase containing one FAD per subunit, which reduces the active site disulfide in oxidized thioredoxin (Trx). The isozymes from mammalian sources vary in molecular mass between 55-67 kDa, compared to 35 kDa enzymes found in prokaryotes, plants, or yeast.[1,2] Mammalian thioredoxin reductase contains a selenocysteine residue, which is essential for the enzymatic activity.[3,4] The mammalian enzyme has a much broader substrate specificity than the prokaryotic enzyme. The mammalian enzyme will reduce both mammalian and *E. coli* thioredoxins, as well as non-disulfide substrates such selenite, lipoic acids, lipid hydroperoxides, and hydrogen peroxide.[5]

Thioredoxin reductase is one of the antioxidant enzymes present in the mammalian cell. Together with catalase, glutathione peroxidase, and superoxide dismutase, it helps remove of reactive oxygen species (ROS) from the cell. Hydrogen peroxide, a deleterious oxidant in the cell, is reduced directly by mammalian TrxR.

The basis of nitric oxide (NO) activity is nitrosylation of transition metals or basic amino acids, such as cysteine. Impairment of NO metabolism and nitrosylation has led to the terms nitrosative stress and nitrosants, which are analogous to oxidative stress and oxidants.[5] Glutathione will react with nitrosants to form a S-nitrosoglutathione adduct (GS-NO), which can be cleaved directly by thioredoxin reductase.[6]

This product is supplied as a solution in 50 mM Tris-HCl, pH 7.4, containing 1 mM EDTA, 300 mM NaCl, and 10% glycerol.

Purity: minimum 90% (SDS-PAGE)

Specific Activity**:** 100-300 units per mg protein

Unit Definition: One unit will cause the increase of A_{412} of 1.0 per minute at pH 7.0 at 25 °C (when measured in a non-coupled assay containing DTNB alone as the substrate).

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product is shipped on dry ice and it is recommended to store the product at –20 °C. Avoid freeze-thaw cycles.

Procedure
The assay of thioredoxin reductase (TrxR) uses 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) as a substrate. TrxR catalyzes the following reaction:

$$\text{DTNB} + \text{NADPH} + H^+ \xrightarrow[\text{reductase}]{\text{Thioredoxin}} 2\ \text{TNB} + NADP^+$$

TNB (5'-thionitrobenzoic acid) has a strong yellow color, which can be measured at 412 nm.

Reagents required for assay procedure
- 1 M Potassium phosphate buffer, pH 7.0
- 500 mM EDTA solution, pH 7.5
- 63 mM DTNB (Product No. D 8130) [5,5'-dithio-bis(2-nitrobenzoic acid)] solution, 25 mg/ml in ethanol
- BSA (Product No. A 8022) solution, 20 mg/ml in water
- 48 mM NADPH (Product No. N 6505) solution, 40 mg/ml in water
- Enzyme sample – the reaction rate is linear when 0.02-0.15 unit of thioredoxin reductase is used per reaction

Prepare reaction mixture - 10 ml (enough for twenty reactions):

Solution	Final conc.	Amount
Phosphate Buffer	100 mM	1.00 ml
EDTA	10 mM	0.20 ml
DTNB	5 mM	0.80 ml
BSA	0.2 mg/ml	0.10 ml
NADPH	0.24 mM	0.05 ml
Water		7.85 ml

Reaction scheme

Sample	Enzyme	Water	Reaction mix
control	0	100 µl	500 µl
sample	x µl	100 – x µl	500 µl

The assay is performed at room temperature (25 °C.)

1. Place x µl (0.02-0.15 unit) of the enzyme sample in a 0.5 ml cuvette.
2. Add water to give a volume of 100 µl.
3. Add 500 µl of the reaction mixture to the cuvette and mix by inversion.
4. Monitor the initial rate of formation of the yellow color by measuring the absorbance at 412 nm for approximately 1 minute. An enzymatic program may be used: delay = 5 seconds, interval = 10 seconds, number of readings = 6.
5. Run a control consisting of reagents alone without the enzyme.
6. Calculate the amount of enzyme present.

Calculation

$$\text{Unit/ml} = \frac{(\Delta A_{412}/\text{min}) \times \text{dil} \times 0.6}{(\text{enzvol})}$$

0.6 = assay volume
ΔA_{412}/min = difference between sample and control absorbance readings per minute
enzvol = volume of enzyme sample in ml
dil = sample dilution factor

Note: The activity of thioredoxin reductase may also be determined using mammalian thioredoxin (Product No. T 8690; 3 µM) and bovine insulin (Product No. I 5500; 174 µM) as substrates.[5] The activity of this reaction is determined by measuring the oxidation of NADPH during the reaction and the activity is similar to the value obtained with DTNB. One unit of thioredoxin reductase activity using mammalian thioredoxin and bovine insulin as substrates is defined as one micromole of NADPH oxidized per minute. To convert from DTNB units to NADPH units, use the following formula:

$$\text{one NADPH unit} = \frac{\text{DTNB units}}{13.6 \times 2}$$

where 13.6 is the millimolar extinction coefficient (E^{mM}) of DTNB of which 2 moles are produced per mole of NADPH oxidized.

References

1. Holmgren, A., and Bjornstedt, M., Methods in Enzymol., **252**, 199-208 (1995).
2. Williams, C.H., et al., Eur. J. Biochem., **267**, 6110-6117 (2000).
3. Fujiwara, N., et al., Biochem. J., **340**, 439-444 (1999).
4. Zhong, L., and Holmgren, A., J. Biol. Chem., **275**, 18121-18128 (2000).
5. Arner, E.S.J., et al., Methods in Enzymol., **300**, 226-239 (1999).
6. Nordberg, J., and Arner, E.S.J., Free Radical Biology and Medicine, **31**, 1287-1312 (2001).

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